# Sortase A

### Week 7

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

## Summary

# 1 PCR of GB1-Sortase A (GS) with new primers

## Responsible

Aman Mebrahtu, Bethel Tesfai Embaie and Reskandi Rudjito

## Aim

To amplify GS and add suffix

## Protocols used

Q5® High-Fidelity 2X Master Mix
Gel purification

## Modifications and comments to protocols

No Modifications were made to the protocol. Two samples were prepared for PCR, resulting in a total number of four samples. The total volume of each reaction was set to 25 $\mu$l. Annealing temperature was set to 66 °C

| | |
|---|---|
| PCR Amplification (Q5): | 1 $\mu$l DNA sample used in PCR mix. |
| PCR Amplification (Q5) primers: | GS_Suffix_R (reverse) |
| | Prefix_F (forward) |

# Experimental Setup

Table 1: Concentration of samples used

| Sample | Initial Concentration [ng/$\mu$l] |
|---|---|
| GS1 | 8 |
| GS2 | 3 |
| GS- | 8 |

# Results and Conclusions

## Gel electrophoresis

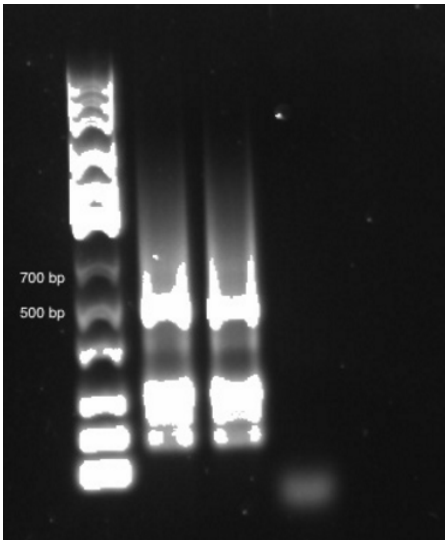


Figure 1: Result of the gel electrophoresis

Table 2: Gene size

| Gene | Size [bp] |
|---|---|
| GS | 662 |
| GS (prefix + suffix) | 677 |

Table 3: Samples in gel 1.

| Lane | Sample | Comment |
|---|---|---|
| 1 | Ladder | - |
| 2 | GS-1 | band of desired size, with some contaminating primer dimers |
| 3 | GS-2 | band of desired size, with some contaminating primer dimers |
| 4 | negative control | contaminants |

PCR worked, as bands of desired size were observed on gel. Due to the formation of primer dimers, gel purification was performed to remove these contaminants from the sample.

## Gel purification

After gel purification the DNA concentration on nanodrop was 3.6 ng/ $\mu$l.

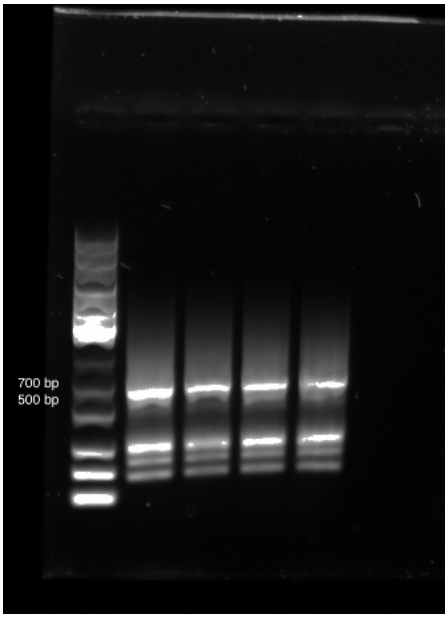


Figure 2: Gel of GS(P+S) for purification

## Discussion and Troubleshooting

GB1-Sortase was successfully amplified with the standard prefix and suffix for the biobrick. The primers, however had some overlapping regions resulting in primer dimer formation and contaminating the sample. Gel purification was performed to remove contaminants.The purified product will be used to ligate into ampicillin backbone.

# 2 Digestion and Ligation of GS and Ampicillin backbone pSB1A3

## Responsible

Aman Mebrahtu, Bethel Tesfai Embaie and Reskandi Rudjito

## Protocols used

Digestion and Ligation
PCR Purification

## Modifications and comments to protocols

Digestion calculations were performed according the initial sample concentrations of insert and backbone. Re-digestion was performed as a back-up.

# Experimental Setup

Table 4: Sample Labelling

| Sample | Abbreviation |
|---|---|
| GS (with prefix and suffix) - 1 | GS (P+S) - 1 |
| GS (with prefix and suffix) - 2 | GS (P+S) - 2 |
| Ampicilin Backbone pSB1A3 | AB |

## Double Digestion

Table 5: Concentration of digested samples

| Sample | Digested product (ng/ $\mu$l ) |
|---|---|
| GS (with prefix and suffix) - 1 | 3.6 |
| Backbone | 2.6 |

Table 6: Enzyme solution stock (for DNA around 10 ng)

| Component | Volume( $\mu$l ) |
|---|---|
| Enzyme | 1 |
| Buffer | 10 |
| Sterile water | 89 |

Table 7: Volumes of each component used in the digestion

| Reaction | Volume (20 $\mu$l) | Volume (10 $\mu$l) |
|---|---|---|
| DNA | 1 | 0.5 |
| Buffer SH 10X | 2 | 1 |
| EcoRI | 1 | 0.5 |
| PstI | 1 | 0.5 |
| $ddH_2O$ | 12.6 | |
| **PCR purified product** | **2 ng/ul** | |

## Re-digestion (backup)

Table 8: Volumes of each component used in the re-digestion

| Reaction | Volume (20 $\mu$l) |
|---|---|
| DNA | 5 |
| Buffer SH 10X | 2 |
| EcoRI | 0.2 |
| PstI | 0.2 |
| $ddH_2O$ | 12.6 |
| **PCR purified product** | **1.2 ng/ul** |

## Ligation

Table 9: *the table shows a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes (variable)

| ***Ligation with T4 Ligase (NEB)*** | | |
|---|---|---|
| **COMPONENT** | **20 l REACTION** | **Volume ()l** |
| 10X T4 DNA Ligase Buffer* | 2 l | 2 |
| Vector DNA (2.2 kb) | 2.6 ng (0.020 pmol) | 1 |
| Insert DNA (677 b) | 2.4 ng | 1,2 |
| Nuclease-free water | to 20 l | 14,8 |
| T4 DNA Ligase | 1 l | 1 |

## Results and Conclusions

Desired PCR product of ligated GS (P+S) is 942 bp.
Conc. of ligated product is 953 ng/ul (nanodrop); but wrong blank was used.

## Discussion and Troubleshooting

Ligated products were stored for transformation into both Top10 and BL21 cells the following day.

# 3 Transformation of pSB1A3-GS in Top10 and BL21 cells

## Responsible

Bethel Tesfai Embaie and Reskandi Rudjito

## Protocols used

Transformation

# Results and Conclusions

The transformation was unsuccessful as no colonies were seen.

# Discussion and Troubleshooting

There are two reasons for an unsuccessful transformation in this case. One reason could be that the ligation was unsuccessful and therefor the cells could not survive. Another reason could be that some mistakes were made when following the Transformation protocol.

Table 10: General Parameters

| | | |
|---|---|---|
| **Concentration DNA sample 1** | 1,2 | ng/ul |
| **Ratio of vector:insert 1 (molar!)** | 3 | x more insert |
| **Concentration DNA sample 2** | 0 | ng/ul |
| **Ratio of vector:insert 2 (molar!)** | 3 | x more insert |
| **Concentration DNA vector** | 12,7 | ng/ul |
| **Size of vector (bp)** | 2200 | bp |
| **Amount of vector per reaction** | 50 | ng |
| **Amount of vector per reaction (mol)** | 0,00000000000003678 | moles |
| **Amount of vector per reaction** | 3,94 | ul |
| **Amount of T4 DNA Ligase** | 0,5 | ul |
| **Total reaction volume** | 30 | ul |

# 4 Re-ligation of and Transformation GS (Sortase with prefix and suffix)

## Responsible

Bethel Tesfai Embaie and Reskandi Rudjito

## Protocols used

Digestion and Ligation
Transformation

## Modifications and comments to protocols

Transformation was performed according to protocol
Transformation done on Top10 and BL21

# Experimental Setup

Two sets of digestions were prepared separately labeled as follows:
Digested GS 1 (Aman)
Digested GS 2 (Reskandi)

# Results and Conclusions

The gel figure is missing.

Table 11: Concentration of the pSB1C3-Nuc PCR product, measured with NanoDrop at 280 nm.

| Sample | Concentration [ng/$\mu$l] |
|---|---|
| Nuc 1.1.1 GS | 33.9 |
| Nuc 1.1.2 GS | 42.6 |

## Discussion and Troubleshooting

The concentration of the PCR products were not high enough to use in further experiments.

# 5 Amplification of GS ligated product

## Responsible

Aman Mebrahtu and Reskandi Rudjito

## Protocols used

PCR Amplification (Q5)

## Modifications and comments to protocols

No changes were made in the protocols

# Experimental Setup

The samples that were amplified:
GS1 Ligation Product
GS2 Ligation Product

Table 12: Volumes for each component in the PCR reaction mix.

| Component | 12.5 $\mu$l Reaction | Final Concentration |
|---|---|---|
| Q5 High-Fidelity 2X Master Mix | 6.25 | 1X |
| 10 $\mu$lM Forward Primer | 0.625 | 0.5$\mu$M |
| 10 $\mu$lM Reverse Primer | 0.625 | 0.5$\mu$M |
| Template DNA | 0.5 | 1 ng |
| Nuclease-Free Water | 4.5 | |

Table 13: Thermocycling Conditions for a Routine PCR

| STEP | TEMP | TIME |
|---|---|---|
| Initial denaturation | 98 | 30 seconds |
| 25-35 Cycles | 98 | 10 seconds |
| | 52 | 30 seconds |
| | 72 | 20 seconds |
| Final extension | 72 | 2 minutes |
| Hold | 4-10 | |

# 6 PCR of GS-1

## Responsible

Reskandi Rudjito and Bethel Tesfai Embaie

## Protocols used

PCR Amplification (Q5)

## Modifications and comments to protocols

No changes were made in the protocols

# Experimental Setup

The samples that was amplified:
purified GS (8 ng/$\mu$l)

Table 14: Volumes for each component in the PCR reaction mix.

| Component | 25 $\mu$l Reaction | 50 $\mu$l Reaction | Final Concentration |
|---|---|---|---|
| Q5 High-Fidelity 2X Master Mix | 12.5 | 25 | 1X |
| 10 $\mu$lM Forward Primer | 1.25 | 2.5 | 0.5$\mu$M |
| 10 $\mu$lM Reverse Primer | 1.25 | 2.5 | 0.5$\mu$M |
| Template DNA | 1 | 2 | 1 ng |
| Nuclease-Free Water | 9 | up to 50 | |

Table 15: Thermocycling Conditions for a Routine PCR

| STEP | TEMP | TIME |
|---|---|---|
| Initial denaturation | 98 | 30 seconds |
| 25-35 Cycles | 98 | 10 seconds |
| | 58 | 30 seconds |
| | 72 | 20 seconds |
| Final extension | 72 | 2 minutes |
| Hold | 4-10 | |

# Results and Conclusions

The PCR product was purified and nanodropped, resulting in a concentration of: 18.9 ng/$\mu$l However, the gel of PCR product showed no clear band.

# Discussion and Troubleshooting

PCR condition was still not optimized. The wrong template was used (without the complete suffix). A second PCR was to be run to amplify GS-2.

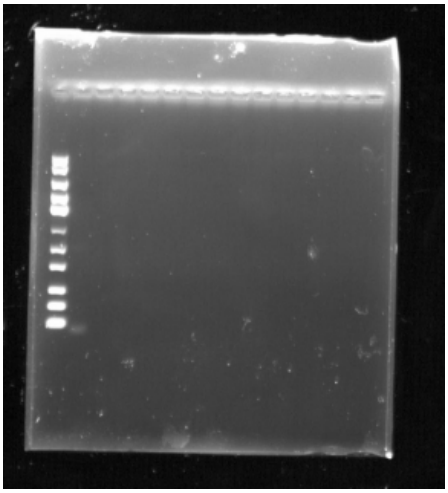

Figure 3: Gel of PCR product GS-1

# 7 PCR of GS-2

## Responsible

Reskandi Rudjito and Bethel Tesfai Embaie

## Protocols used

PCR Amplification (Q5)

## Modifications and comments to protocols

No changes were made in the protocols

# Experimental Setup

The samples that was amplified:
purified GS with suffix (3.6 ng/$\mu$l)

Table 16: Volumes for each component in the PCR reaction mix.

| Component | 25 $\mu$l Reaction | 6.5 $\mu$l Reaction | Final Concentration |
|---|---|---|---|
| Q5 High-Fidelity 2X Master Mix | 12.5 | 3.125 | 1X |
| 10 $\mu$lM Forward Primer | 1.25 | 0.3125 | 0.5$\mu$M |
| 10 $\mu$lM Reverse Primer | 1.25 | 0.3125 | 0.5$\mu$M |
| Template DNA | 1 | 0.5 | 1 ng |
| Nuclease-Free Water | 9 | 2.25 | |

Table 17: Thermocycling Conditions for a Routine PCR

| STEP | TEMP | TIME |
|---|---|---|
| Initial denaturation | 98 | 30 seconds |
| 25-35 Cycles | 98 | 10 seconds |
| | 58 | 30 seconds |
| | 72 | 20 seconds |
| Final extension | 72 | 2 minutes |
| Hold | 4-10 | |

## Results and Conclusions

The gel of PCR product shows a band of the expected size.

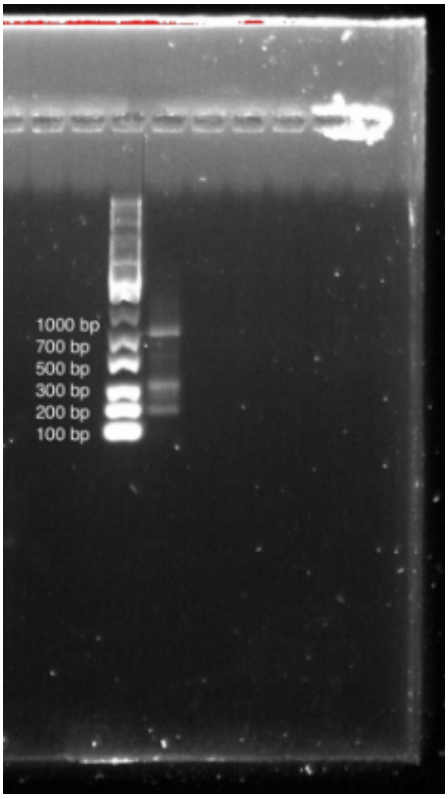


Figure 4: Gel of PCR product GS-2

## Discussion and Troubleshooting

Desired product of around 700 bp was obtained, however some unspecific bands of possible primer dimers may have also been formed. (The ladder did not run smoothly when the gel was used for the second time)

# 8 Digestion, Ligation and Transformation of GS

## Responsible

Reskandi Rudjito and Bethel Tesfai Embaie

## Protocols used

Digestion and Ligation
Transformation with TOP10

## Modifications and comments to protocols

# Experimental Setup

The samples used:
new GS (18.9 ng/$\mu$l )
pSB1C3 Chloramphenicol backbone (25 ng/$\mu$l )

## Digestion I

Table 18: Enzyme Master Mix

| Component | Volumes [$\mu$l] |
|---|---|
| Buffer SH 10X | 2 |
| EcoRI | 0.2 |
| PstI | 0.2 |
| Sterile water | 6.5 |

Table 19: Digestion reaction of New GS

| Component | Volumes [$\mu$l] |
|---|---|
| DNA GS (18.9 ng/$\mu$l ) | 5 |
| Mastermix | 5 |

Table 20: Digestion reaction of pSB1C3

| Component | Volumes [$\mu$l] |
|---|---|
| DNA (25 ng/$\mu$l ) | 4 |
| Buffer SH 10X | 1 |
| EcoRI | 0.2 |
| PstI | 0.2 |
| Sterile water | 4.6 |

All samples were PCR purified, binding to column was repeated 6x, concentration was measured using nanodrop. The final concentration of purified digested GS was 6.5 ng/$\mu$l.

Table 21: General parameters

| | | |
|---|---|---|
| Concentration DNA sample 1 | 6,5 | ng/$\mu$l |
| Ratio of vector:insert 1 (molar!) | 3 | x more insert |
| Concentration DNA sample 2 | 0 | ng/$\mu$l |
| Ratio of vector:insert 2 (molar!) | 0 | x more insert |
| Concentration DNA vector | 12,7 | ng/$\mu$l |
| Size of vector (bp) | 2200 | bp |
| Amount of vector per reaction | 50 | ng |
| Amount of vector per reaction (mol) | 0,00000000000003678 | moles |
| Amount of vector per reaction | 3,94 | $\mu$l |
| Amount of T4 DNA Ligase | 0,5 | $\mu$l |
| Total reaction volume | 20 | $\mu$l |

## Ligation I

The digested insert GS was ligated into the digested Chloramphenicol backbone.

Table 22: Amount of DNA from insert GS and pSB1C3 Backbone

| Size of insert (bp) | Volumes [$\mu$l] |
|---|---|
| GS 669 | 7.02 |
| pSB1C3 2200 | 3.94 |

Table 23: Ligation reaction mix

| **Reaction 1 (Sample 1 + Vector)** | **20 uL reaction** | **10 uL reaction** |
|---|---|---|
| insert GS | 7.02 | 3.51 |
| pSB1C3 backbone | 3.94 | 1.97 |
| T4 Ligase | 0.5 | 0.25 |
| 10x T4 DNA Ligase Buffer | 2 | 1 |
| ddH20 | 6.55 | 3.27 |

## Digestion II

A second digestion of New GS 2 was performed.

Table 24: Digestion reaction of New GS 2

| Component | Volumes [$\mu$l] |
|---|---|
| DNA (20 ng/$\mu$l ) | 2 |
| Buffer SH 10X | 1 |
| EcoRI | 0.5 |
| PstI | 0.5 |
| Sterile water | 6 |

The digested product was PCR purified, resulting in a concentration of 6.9 ng/$\mu$l.

## Ligation II

The new digested insert GS 2 was ligated into the digested Chloramphenicol backbone. The ligated products were transformed in TOP10 cells and incubated overnight.

Table 25: General parameters

| | | |
|---|---|---|
| Concentration DNA sample 1 | 6.9 | ng/$\mu$l |
| Ratio of vector:insert 1 (molar!) | 4 | x more insert |
| Concentration DNA sample 2 | 0 | ng/$\mu$l |
| Ratio of vector:insert 2 (molar!) | 0 | x more insert |
| Concentration DNA vector | 12,7 | ng/$\mu$l |
| Size of vector (bp) | 2200 | bp |
| Amount of vector per reaction | 50 | ng |
| Amount of vector per reaction (mol) | 0,00000000000003678 | moles |
| Amount of vector per reaction | 3.94 | $\mu$l |
| Amount of T4 DNA Ligase | 0.5 | $\mu$l |
| Total reaction volume | 20 | $\mu$l |

Table 26: Amount of DNA from insert GS 2 and pSB1C3 Backbone

| Size of insert (bp) | Volumes [$\mu$l] |
|---|---|
| GS 669 | 8.81 |
| pSB1C3 2200 | 3.94 |

Table 27: Ligation reaction mix

| **Reaction 1 (Sample 1 + Vector)** | **20 uL reaction** | **10 uL reaction** |
|---|---|---|
| insert GS 2 | 8.81 | 4.41 |
| pSB1C3 backbone | 3.94 | 1.97 |
| T4 Ligase | 0.5 | 0.25 |
| 10x T4 DNA Ligase Buffer | 2 | 1 |
| ddH20 | 4.75 | 2.37 |

## Results and Conclusions

A gel was run before ligation, to confirm all the right samples were used.There was, however no band at the expected size of 687 bp.


Figure 5: Result of the gel electrophoresis of samples with 1 % agarose at 100 V. DNA Ladder (1) New GS

The PCR purified digested products, were of high enough concentration to be used for ligation.

Digested GS 1: 6.5 ng/$\mu$l
Digested GS 2: 6.9 ng/$\mu$l .

## Discussion and Troubleshooting

For digestion, digest 100 ng of DNA. When doing PCR purification, repeat the DNA binding step 6x to increase the amount of DNA elluted.

# 9 PCR to confirm the ligation of GS-2

## Responsible

Reskandi Rudjito and Bethel Tesfai Embaie

## Protocols used

PCR Amplification (Q5)

## Modifications and comments to protocols

No changes were made in the protocols

# Experimental Setup

The samples that was amplified:
purified GS with suffix (3.6 ng/$\mu$l)

Table 28: Volumes for each component in the PCR reaction mix.

| Component | 25 $\mu$l Reaction | 6.5 $\mu$l Reaction | Final Concentration |
|---|---|---|---|
| Q5 High-Fidelity 2X Master Mix | 12.5 | 3.125 | 1X |
| 10 $\mu$lM Forward Primer | 1.25 | 0.3125 | 0.5$\mu$M |
| 10 $\mu$lM Reverse Primer | 1.25 | 0.3125 | 0.5$\mu$M |
| Template DNA | 1 | 0.5 | 1 ng |
| Nuclease-Free Water | 9 | 2.25 | |

Table 29: Thermocycling Conditions for a Routine PCR

| STEP | TEMP | TIME |
|---|---|---|
| Initial denaturation | 98 | 30 seconds |
| 25-35 Cycles | 98 | 10 seconds |
| | 52 | 30 seconds |
| | 72 | 20 seconds |
| Final extension | 72 | 2 minutes |
| Hold | 4-10 | |

# Results and Conclusions

The gel of samples show that digestion was successful whereas ligation was unclear. GS digested product shows a faint band of the correct expected size. GS ligation product shows a faint band that looks like digestion product but ligation isn't visible. For PCR product of ligated GS, it is unclear why bands of this size appear. Ligation product might also have been amplified, leading to faint band around 700 bp.

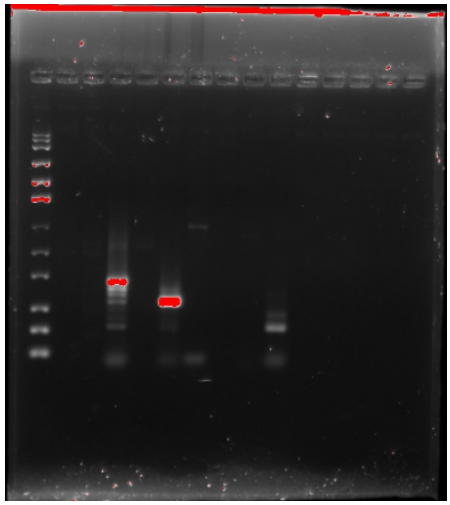

Figure 6: Result of the gel electrophoresis of the PCR amplification product with 1 % agarose at 100 V. (1) DNA Ladder (2) GS digested (3) GS ligation product (4) GS PCR of ligation product

## Discussion and Troubleshooting

Several samples indicate that digestion was successful whereas ligation couldn't be seen in any of the samples. It is likely that even though for this ligation, the buffer was carefully vortexed, the ligation could not be performed succssfully. A solution to use new ligase from Sigma, to improve the efficacy of PCR reactions.